REMARKS

Patentability Under 35 USC § 112, first paragraph

2. Claims 2, 5, and 48 stand rejected under 35 USC § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. More specifically, Claim 2 was rejected for not conveying to one of skill in the art at the time the application was filed that Applicants had possession of the invention as claimed.

At the time the application was filed, Applicants had possession of the invention of Claim 2, as amended. Citing reasons of the history of biological deposits for patent purposes, the goals of the patent law, and the practical difficulties of describing unique biological materials in a written description, the C.A.F.C. has held that reference in the specification to a deposit in a public depository, which makes its contents accessible to the public when it is not otherwise available in written form, constitutes an adequate description of the deposited material sufficient to comply with the written description requirement of Section 112, paragraph 1. (Enzo Biochem, Inc. v. Gen-Probe Inc.("Enzo II"), *296 F.3d 1316; 63 USPQ2D 1609, July 15, 2002*). By means of biological deposits made under the terms of the Budapest Treaty (as evidenced in the specification at page 5, line 21 through page 6, line 2 and in the depository receipts), Applicants provided a written description of three isolated nucleic acids (contained in pSW91, pnit4, and pnitex2) that encode claimed nitrilase enzyme.

The Enzo II opinion also adopted provisions from the Guidelines issued by the USPTO that state that the written description requirement can be met by a functional description of claimed materials, if coupled with a known or disclosed correlation between function and structure. Claim 2 sets out a "structure" by referencing SEQ ID NO:5 and (by previous amendment) requiring nitrilase functionality or activity for the composition. Applicants support the nitrilase functionality with the spectro-photometric assay and data described in Example 15, and in the specification at page 31, lines 8-13, with a test for nitrilase activity (indicated by the ability to catalyze the conversion of MGN to 4-CPA). All of Applicants' expression systems (SS1001, SS1002, SW90, and SW91) catalyzed this reaction.

Applicants have also supplied the sequences of primers derived from conserved regions of bacterial nitrilase sequences (SEQ ID NOs.:1, 2) and the sequences of reverse and forward primers (SEQ ID NOs.:6, 7, 8, 9, 10, 11, 12) used to amplify the

nitrilase coding sequence from *A. facilis* 72W genomic DNA and useful in the construction of claimed materials (SEQ ID NOs.:17-32). With deduced (SEQ ID NOs.:5 and 14), synthetic (SEQ ID NO.:16), and actually determined (SEQ ID NOs.:3, 4, 13, 15) sequences, Applicants have provided written descriptions of species of nitrilase enzyme sequences. Support for the sequences is found in the Sequence Listing that forms a portion of the specification and is sufficient to be representative of the entire scope of the claimed subject matter.

Applicants have canceled Claims 5 and 48 for reasons set out at page 9 that are unrelated to compliance with 35 USC § 112, first paragraph, and traverse the rejection of Claim 2 as currently amended. New Claim 49, setting out a more preferred embodiment of the nucleic acid, is added.

Reconsideration of Claim 2, consideration of New Claim 49, withdrawal of the rejection, and prompt allowance of the claims are respectfully requested.

Patentability Under 35 USC § 112, second paragraph

3. Claims 2, 5 and 48 stand rejected under 35 USC § 112, second paragraph, for indefiniteness with regard to the percent identity parameter in the claims of 71 %.

As stated above, Applicants have cancelled Claims 5 and 48 for reasons unrelated to compliance with 35 USC 112, second paragraph and traverse the rejection of Claim 2 as currently amended. New Claim 49 is added.

Claim 2 as filed referred to a 71 % identity as determined by the described algorithm. The sequence comparison to cited prior art (the nucleic acid sequence that encodes for the *Rhodococcus rhodochrous* K22 nitrilase) was performed relative to that of the *E. coli* SS1001 nitrilase sequence, yielding a percent identity of 70.732.

Applicants have amended Claim 2 to claim an isolated nucleic acid fragment comprising a first nucleotide sequence encoding a polypeptide with nitrilase activity having at least 80 % identity as determined by the Needleman and Wunsch algorithm under default parameters compared to a polypeptide encoded by the sequence set out in SEQ ID NO:5. New Claim 49 is drawn to an isolated nucleic acid fragment having at least 90% identity with the sequence set out in SEQ ID NO:5. The specification clearly provides the default parameters for these claimed levels of % identity at page 26, lines 6-13.

In light of the amendments and remarks, reconsideration of Claim 2, consideration of new Claim 49, withdrawal of the rejection, and prompt allowance of the claims are respectfully requested.

Patentability Under 35 USC § 102(b)

4. Claims 1-3, 5, 9, 11, 13, 15, and 48 stand rejected under 35 USC § 102(b) as being anticipated by Kobayashi *et al.* (*Biochemistry* 31:9000-9007 (1992)).

Applicants have amended Claim 2 to claim an isolated nucleic acid fragment comprising a first nucleotide sequence encoding a polypeptide with nitrilase activity having at least 80 % identity as determined by the Needleman and Wunsch algorithm under default parameters compared to a polypeptide encoded by the sequence identified in SEQ ID NO:5. A new Claim 49 is added to an isolated nucleic acid fragment comprising a first nucleotide sequence encoding a polypeptide with nitrilase activity having at least 90 % identity as determined by the Needleman and Wunsch algorithm compared to a polypeptide encoded by the sequence identified in SEQ ID NO:5. Claims 5 and 48 have been cancelled.

A rejection for anticipation under 35 USC 102(b) requires that all elements of the claimed invention be found in the cited reference. Applicants strongly disagree that "Here, the only difference between the products is the claimed method of making". Applicants maintain that the composition of Kobayashi (the nucleic acid sequence that encodes for the *Rhodococcus rhodochrous* K22 nitrilase) and the compositions of amended Claims 1-3, 5, 9, 11, 13, 15, and 48 (nucleic acid sequences that encode for the *Acidovorax facilis* 72W nitrilase) are not the same. This position is supported by the fact that the nitrilase enzyme encoded by the gene sequence reported by Kobayashi *et al.* has markedly different enzyme activities and temperature stability as compared to the nitrilase enzymes encoded by the nucleic acid fragments claimed in the present application.

Kobayashi *et al.* (*Tetrahedron* (1990) 46:5587-5590) reported on page 5589 that: "In the present study, the *Rhodococcus* K22 nitrilase was capable of hydrolyzing only one cyano group of aliphatic dinitriles to a carboxylic acid group." In this study, glutaronitrile was hydrolyzed quantitatively to 4-cyanobutyric acid (no detection of glutaric acid), and adiponitrile was quantitatively hydrolyzed to 5-cyanovaleric acid (no production of adipic acid). In contrast, Gavagan *et al.* (*J. Org. Chem.* 63:4792-4801 (1998)) reported that *Acidovorax facilis* 72W nitrilase (a nitrilase encoded by the nucleic acid fragments of the present application) hydrolyzed glutaronitrile to both 4-

cyanobutyric acid (92 % yield) and glutaric acid (8 % yield), and hydrolyzed adiponitrile to produce 5-cyanovaleric acid in less than 50 % yield, with adipic acid being the major hydrolysis product.

In a separate publication, Kobayashi *et al.* (*J. Bacteriology* (1990) 4807-4815) report the substrate specificity of the *Rhodococcus rhodochrous* K22 nitrilase (page 4813, Table 3). Gavagan *et al.* (*Appl. Microbiol. Biotechnol.* (1999) 53: 654-659 in Table 1, page 656) report the substrate specificity of *Acidovorax facilis* 72W nitrilase. In comparing these two reports, it is clear that these two nitrilase enzymes have completely different substrate specificities. For example, the relative % activity of succinonitrile and crotononitrile as substrates with K22 nitrilase are 271% and 100 %, respectively; in contrast, there is no detectable activity for conversion of crotononitrile with 72W nitrilase when compared to succinonitrile, which is a substrate. Similarly, the relative % activity for valeronitrile and fumaronitrile as substrates with K22 nitrilase are 27.4 % and 40.8 % respectively; in contrast, there is no detectable activity for conversion of valeronitrile with 72W nitrilase when compared to fumaronitrile, which is the most active substrate listed in Table 1 for hydrolysis by 72W nitrilase. It is clear from comparing the data in the two tables that the K22 nitrilase has a markedly different substrate specificity when compared to 72W nitrilase, and that unlike K22 nitrilase, the 72W nitrilase does not readily hydrolyze aliphatic mononitriles such as butyronitrile, valeronitrile, and capronitrile. Instead, the 72W nitrilase shows a clear preference for the hydrolysis of aliphatic dinitriles that is not exhibited by the K22 nitrilase.

Kobayashi *et al.* (*J. Bacteriology* (1990) 4807-4815) also report the temperature stability of *Rhodococcus rhodochrous* K22 nitrilase (page 4811, last paragraph). After preincubation at 50 °C for one hour, followed by assay at 25 °C, the K22 nitrilase had only 6.7 % of its initial activity. In marked contrast, Gavagan *et al.* (*Appl. Microbiol. Biotechnol.* (1999) 53: 654-659, page 655, last paragraph) report, that there was no loss of activity of 72W nitrilase after heating for one hour at 50 °C. Kobayashi *et al.* report a 28 % recovery of K22 nitrilase activity after preincubation for one hour at 45 °C, while Gavagan *et al.* report that the 72W nitrilase takes 14.6 days to lose 50 % of its activity at 45 °C. The temperature stabilities of these two nitrilases are markedly different.

The vague similarities of the Kobayashi nucleic acid sequence encoding K22 nitrilase to the claimed nucleic acid sequences cited by the examiner (where the cited K22 nucleic acid sequence has only a 71.274 % homology to SEQ ID NO: 5) do not prove

that "the only difference between the products is the claimed method of making" and are insufficient to uphold a rejection for anticipation. It is clear that the Kobayashi nucleic acid sequence is not identical to the claimed sequences, nor does the sequence encode a nitrilase activity with even remotely similar substrate specificity, regioselectivity, or thermal stability.

In addition to the specific sequences provided in the specification, Applicants have defined sequence variations that are also operable in the invention. These additional factors (i.e., stringent hybridization, a functional property, and the deposit of biological material, demonstrated nitrilase activity) together further support the novelty of the claimed invention.

In light of the comments and amendments, Applicants respectfully request reconsideration of the claims, withdrawal of the rejection, and prompt allowance of Claims 1-3, 9, 11, 13 and 15.

Patentability Under 35 USC § 103(a)

6., 7. The Examiner is correct in the presumption that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made.

8. Claims 16-19 stand rejected under 35 USC 103(a) as being unpatentable over Kobayashi in view of Anderson et al. (US 5,935,840).

9. Claim 46 stands rejected under 35 USC 103(a) as being unpatentable over Kobayashi in view of Anderson et al. (US 5,935,840) and further in view of Galen (US 6,413,768).

The Examiner finds that Kobayashi teaches the limitations of Claims 1-3, 5, 9, 11, 13, 15, and 48. However, Kobayashi does not teach the use of chromosomally integrated vectors and ribosome binding sites. The Examiner finds that Anderson teaches the use of chromosomally integrated vectors and ribosome binding sites at column 5, lines 23-38). Kobayashi in view of Anderson do not teach the specific stains listed in Claim 46. However, the Examiner finds that Galen teaches MG1655.

Applicants have claimed a novel composition. Applicants have shown in Section 7 that the Kobayashi and Applicants' materials as now claimed possess distinct differences in sequence and properties. In short, the cited Kobayashi reference is deficient as a reference in these rejections for obviousness. As Kobayashi is the

foundation for the rejections of Claims 16-19 and 46, if that reference is removed, the rejection must fail.

Even with those differences, combining the claimed genetic material with chromosomally integrated vectors and binding sites may have been obvious to try. However, it was not obvious from the cited references that the combination of the novel genetic material and the chromosomally integrated vectors and ribosome binding sites (Anderson) would be operable. Likewise, the use of M61655 (Galen) as a host cell may have been obvious to try, but the operability of that combination of references was also not obvious.

Having traversed the rejections of Claims 16-19 and 46, Applicants respectfully request the reconsideration of these claims, withdrawal of the rejections, and prompt allowance of the claims.

Additional Amendments

Claims 5 and 48 are cancelled, the subject matter properly addressed in the remaining claims without further reference to the source of the genetic material.

Allowable Subject Matter

10. Claims 4, 10, 12, 14, 20, and 47 are objected to as including subject matter which was non-elected and which must be deleted prior to indication that the claims are allowable. Additionally, Claim 14 is objected to as being dependent upon a rejected base claim.

Applicants have respectfully amended Claim 47 to include only the elected restriction subgroup (not species) SEQ ID NO:5 (to nucleic acid) and removed non-elected subject matter.

Additionally, Applicants note that Claim 10, as ameded, is drawn to the plasmid pnitex2 contained in SS1001 designated ATCC PTA-1177. This text was inadvertently left out of the claim as originally filed. Support for amendment is found in original Claim 20 and at page 5, line 25, and at Table 2: "Nitrilase Activity in *E. coli* transformants".

Reconsideration of the Claims, withdrawal of the objection, and the prompt allowance of Claims 4, 10, 12, 14, 20, and 47 is respectfully requested.

In light of the Remarks and amendments proposed herein, reconsideration of the Claims, withdrawal of the objection, and the prompt allowance of the claims under prosecution are respectfully requested.

Should any issue remain unresolved, please contact the undersigned.

Respectfully submitted,

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Dated: 6 June 2003

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